Acetyl CoA Synthase
August 24, 2004

Summary

Acetyl CoA synthase catalyzes the conversion of acetate, ATP and coenzyme A into acetyl CoA and AMP. This assay involves the chemical reaction between acetyl CoA and hydroxylamine which liberates CoASH and hydroxamic acid which can be measured colorimetrically.

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Solutions Required

1. 200 mM Tris HCl buffer (FW 157.6)
   (3.15 g/100 mL solution)
   adjust to a pH of 8.1 with 20% KOH
   can be stored in stock

2. 100 mM $MgCl_2 \cdot 6H_20$ (FW 203.3)
   (2.03 g/100 mL solution)
   can be stored in stock

3. 400 mM sodium acetate trihydrate (FW 138.08)
   (5.44 g/100 mL solution)
   can be prepared in stock solution, stored in refrigerator.

4. 3.33 mM CoA Lithium salt (FW 767.5)
   (2.56 mg/mL solution)
   Sigma catalog #C3019
   must be prepared fresh

5. 100 mM ATP disodium salt (FW 551.5)
   (55.15 mg/mL solution)
   Sigma catalog #A2383
   must be prepared fresh

6. 2.5 g $FeCl_3$ in 100 mL 2.0M HCl
   can be stored in stock

7. 1.0M hydroxylamine HCl (FW 69.49)
   mix equal volumes of 2M $NH_2OH \cdot HCl$ (139 mg/mL) and 2M KOH (56.1 mg/mL)
   Sigma catalog #H2391
   must be prepared fresh (or frozen stock)

8. 10 mM lithium potassium acetyl phosphate (FW 184.1)
   (1.84 mg/mL solution)
   Sigma catalog #A0262
   must be prepared fresh

9. 1M KF (FW 58.10)
   (58.10 mg/mL solution)
   Sigma catalog #60238
   can be stored in stock

10. 200 mM glutathione (reduced) neutralized with KOH to pH 4.5
    (61.46 mg/mL solution)
    Sigma catalog #G6529
    can be prepared in stock solution, stored in refrigerator.

Preparation of Cell Extract

Follow general protocol **Preparation of Cell Extract**.

1. After first pelletization of cells, resuspend at 4°C in 20 mM Tris HCl pH 8.1 with 20% glycerol.
2. After second pelletization of cells, resuspend at 4°C in 20 mM Tris HCl pH 8.1 with 20% glycerol.

Procedure

1. For each solution to be assayed, prepare the following solutions in a small test tube:

Solution	Volume (μL)	
	experimental	blank
Tris HCl	250	250
hydroxylamine	100	100
$MgCl_2$	50	50
sodium acetate	50	50
coenzyme A	100	0
ATP	100	100
water	150	250
glutathione	50	50
KF	50	50

2. Prepare the following lithium acetyl phosphate (LAP) standards:

Standard Concentration	Volume (μL)			
	DI water	LAP	hydroxylamine	tris HCl
2.0 μmol/mL	450	200	100	250
1.0 μmol/mL	550	100	100	250
0.6 μmol/mL	590	60	100	250
0.3 μmol/mL	620	30	100	250
0	650	0	100	250

3. Place the solutions to be assayed (not the standards) in a water bath at 37ºC for 5 minutes.
4. Initiate the reactions by adding 100 μL cell-free extract into each of the test tubes and agitating.
5. Permit the reactions to occur for 20 minutes in the water bath.
6. After 20 minutes, terminate the reaction by adding 1000 μL acidic $FeCl_3$ solution. At this time, also mix the acidic $FeCl_3$ solution with the standard solutions.
7. Centrifuge terminated reaction for at 13000 rpm for 10 minutes to pellet precipitated protein
8. Measure the absorbance of each solution in a spectrophotometer at 520 nm.

† Volume of cell extract may be adjusted. Any change in cell extract should be accompanied by a change in the DI water in the cocktails. (For example, if 50 μL of cell extract were used in the experimental, then 300 μL of DI water would be used.) If a sample volume below 50 μL is required to obtain a result within range of the standards, the sample should be diluted prior to mixing with the reagents. For example, in order to dilute by a factor of 10, combine 900 μL of DI water with 100 μL of sample. Then use 100 μL of this diluted solution for the enzyme assay.

Calculation of Activity

One unit (U) of acetyl CoA synthase activity is defined as the amount of enzyme required to produce 1.0 μmole of acetyl CoA in one minute.

1. Fit the results of the standards to the equation: Conc (μmol/mL) = m × Abs

2. Activity = $\frac{1000 \times TV \times m \times Abs \times D}{t_R \times V \times CF}$

Activity:	Volumetric Activity (U/L)
Abs:	Absorbance of sample at 520 nm relative to blank.
m:	The best-fitting slope.
TV:	Total volume in cuvette (1000 μL)
V:	Volume of cell extract used (100 μL)
t_R:	Time of reaction (20 minutes)
CF:	Concentration factor of cell extract (e.g., if a 100 mL sample is concentrated to a 5 mL volume for the French Press, then CF=20)
D:	Dilution factor for cell extract used in the reaction mixture (e.g., if the cell extract is diluted by a factor of 10 prior to being mixed with the reagents, then D = 10)

3. Specific Activity = $\frac{Activity}{Protein\ Concentration}$

Activity:	Volumetric Activity, as calculated in #2 above (U/L)
Protein Concentration:	Protein concentration, as calculated in protocol **Total Protein Concentration** (mg/L)
Specific Activity:	(U/mg protein)

References

T. D. K. Brown, M. C. Jones-Mortiner, H. L. Kornberg (1977) The enzymatic interconversion of acetate and acetyl-coenzyme A in *Escherichia coli*," J. Gen. Microbiol. 102, 327-336.

M. E. Jones, F. Lipmann (1955) Aceto-CoA-Kinase. Methods in Enzymology 1, 585-595.